PHENYLMERCURY TRANSPORT MEDIATED BY *merT-merP* GENES OF *PSEUDOMONAS* K-62 PLASMID pMR26

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The *merB-merA*-deleted plasmid pMRD141 which contains the intact *merT-merP* genes of pMRA17 conferred bacterial hypersensitivity not only to Hg²⁺ but also to C₆H₅Hg⁺. The bacterium with pMRD141 took up significantly more C₆H₅Hg⁺ than its isogenic strain with the cloning vector Bluescript II. The hypersensitivity to C₆H₅Hg⁺ seems to be based on hyperaccumulation of toxic C₆H₅Hg⁺ in the absence of detoxifying enzymes encoded by *merB* and *merA*. Our results show that bacterial transport of C₆H₅Hg⁺ into the cytoplasm is regulated by *merT-merP* genes.

KEY WORDS Phenylmercury transport; *merT-merP*; pMRD141; pMRA17

The broad-spectrum mercury resistance (*mer*) operon determining bacterial resistance to both inorganic and organic mercury has been shown to consist of a regulatory gene (*merR*), an operator/promoter (O/P) region, and at least four structural genes, *merT*, *merP*, *merA*, and *merB*.¹⁻⁴⁾ The *merB* gene encoding organomercurial lyase which cleaves the C-Hg bond of organomercury to yield Hg²⁺ is missing in the narrow-spectrum *mer* operon which only confers bacterial resistance to inorganic mercury.⁵⁻⁷⁾ The *merT* and *merP* genes on both broad-spectrum and narrow-spectrum *mer* operons have been implicated in Hg²⁺ transport and been shown to be required for bacterial resistance to Hg²⁺,^{8,9)} because the Hg²⁺ must be transported into the cytoplasm before it can be reduced by mercuric reductase encoded by *merA*. It seems reasonable to expect that transport of organomercury into the cytoplasm is necessary for the organomercurial lyase to act upon it. Whether the transport of organomercury into the cytoplasm is coded for by the *merT* and *merP* genes remains to be confirmed.

In this paper, by comparing the uptake of phenylmercury by cells carrying a *merB-merA*-deleted plasmid, pMRD141, which still contains the intact *merT* and *merP* genes of pMRA17 containing the entire broad-spectrum *mer* operon of *Pseudomonas* K-62¹⁰⁾ and its isogenic cells with cloning vector Bluescript II, we present experimental evidence that transport of phenylmercury is mediated by the *merT* and *merP* genes.

Escherichia coli XL1-Blue with a deletion plasmid pMRD141 which contains the *merR*, O/P, *merT*, and *merP* genes constructed by eliminating *merA* and *merB* genes from pMRA17 was grown in L-broth with 5 μ M phenylmercury.¹¹⁾ The late-log-phase cells were harvested and resuspended in L-broth containing 100 μ g/ml chloramphenicol, 100 μ M EDTA, and 50 μ M ¹⁴C-phenylmercury (30 mCi/mmol) or 5 μ M ²⁰³HgCl₂ (136 mCi/mmol). After incubation at 37°C, aliquots (0.5 ml) were removed periodically and filtered on a Whatman GF/B glass microfiber filter (0.45 μ m).

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After washing the filters three times with 5 ml L-broth, the radioactivity trapped on the filter was measured with a liquid scintillation spectrometer (Aloka LSC-3500). Bacterial resistance to mercurials was determined on Petri dishes according to the method of Foster et al.¹²⁾ The zones of inhibition of growth around the disks were measured after incubation at 37°C for 16 h.

As shown in Fig. 1, plasmid pMRA17 cloned from *Pseudomonas* K-62 pMR26 conferred bacterial resistance not only to Hg^{2+} but also to $\text{C}_6\text{H}_5\text{Hg}^+$. Recently, the pMRA17 *mer* operon has been shown to consist of a *merR* followed by an O/P and then by *merT*, *merP*, *merA*, and *merB*.²⁾ Deletion of the genes corresponding to *merB* and *merA* from pMRA17 not only resulted in almost complete loss of the mercurial resistance phenotype, but also rendered the cells hypersensitive to both Hg^{2+} and $\text{C}_6\text{H}_5\text{Hg}^+$ compared with its isogenic cells carrying the cloning vector Bluescript II, as shown in Fig. 1. The results suggest that the resistance to phenylmercury shown by the cells with pMRA17 is based on the cleavage of the C-Hg bond by organomercurial lyase encoded by *merB* and reduction of the resultant Hg^{2+} to Hg^0 by the mercuric reductase encoded by *merA*.

Fig. 1. Resistance of *E. coli* XL1-Blue carrying pMRA17 (■), pMRD141 (●), or Bluescript II (△) to Hg^{2+} (A) or $\text{C}_6\text{H}_5\text{Hg}^+$ (B). All values are the means of six determinations.

It has been already shown that expression of Hg^{2+} transport functions mediated by *merT-merP* in the absence of mercuric reductase activity renders the host cell hypersensitive to Hg^{2+} based on hyperaccumulation of toxic Hg^{2+} .^{8,9)} In a previous paper, we reported that the *merT-merP* genes were only involved in Hg^{2+} transport but did not participate in the bacterial transport of methylmercury.¹¹⁾

In view of these findings, we attempted to determine whether the *merT-merP* genes are also involved in the transport of $\text{C}_6\text{H}_5\text{Hg}^+$. As shown in Fig. 2, cells with pMRD141 took up appreciably more $^{203}\text{Hg}^{2+}$ and $^{14}\text{C}_6\text{H}_5\text{Hg}^+$ than isogenic cells with the cloning vector Bluescript II. Deletion of the *merT-merP* genes from pMRD141 would result in complete loss of the hypersensitive phenotype, and the *merT-merP*-deleted mutant took up almost the same amounts of $\text{C}_6\text{H}_5\text{Hg}^+$ as did its isogenic cells with Bluescript II (data not shown). These results clearly demonstrate that the *merT-merP* genes are required for phenylmercury transport into the cells, and expression of *merT-merP* in the absence of functional detoxifying enzymes would render the cells hypersensitive not only to Hg^{2+}

but also to $\text{C}_6\text{H}_5\text{Hg}^+$ based on the hyperaccumulation of mercurials. To our knowledge, this is the first report describing the role of *merT-merP* in organomercury transport.

Fig. 2. Uptake of $^{203}\text{Hg}^{2+}$ (A) or $^{14}\text{C}_6\text{H}_5\text{Hg}^+$ (B) by *E. coli* XL1-Blue with pMRD141 (●) or Bluescript II (△). All values are the means of three determinations.

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